Type III Polyketide Synthases Responsible for Phenolic Lipid Synthesis

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Abstract

In various microorganisms, phenolic lipids, consisting of a polar aromatic ring and a hydrophobic alkyl chain, are synthesized by type III polyketide synthases (PKSs). In Azotobacter vinelandii, two type III PKSs, ArsB and ArsC, are responsible for the biosynthesis of alkylresorcinols and alkylpyrones, respectively, which are the major lipids in the cyst membrane. In Streptomyces griseus, SrsA is involved in synthesizing alkylquinones, which confer resistance to β-lactam antibiotics. In Mycobacterium smegmatis, PKS10 is involved in the biosynthesis of alkylquinones, which are proposed to act as electron-shuttling molecules. The phenolic lipid-producing type III PKSs are distributed in a wide

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variety of Gram-positive and Gram-negative bacteria, some fungi, and plants. Thus, phenolic lipids produced by type III PKSs play important, but so far overlooked, roles as minor components in biological membranes and, more importantly, as mobile electron carriers in the respiratory electron transport chain in some bacteria.

1 Introduction

Polyketides form a large family of natural products that are found in bacteria, fungi, and plants, and they exhibit various biological activities. Polyketides are synthesized by polyketide synthases (PKSs). PKSs possess a ketosynthase activity that catalyzes the condensation of extender units onto an acyl starter substrate or a growing polyketide chain. Type III PKSs, which consist of a homodimeric ketosynthase, are the simplest enzymes among the three types (I to III) of PKSs (Austin and Noel 2003). Formerly, it had been thought that type III PKSs were distributed exclusively in plants, and many plant type III PKSs have been discovered and characterized. For example, chalcone synthase catalyzes the formation of naringenin chalcone, an essential precursor of flavonoids and isoflavonoids, which have attracted significant attention because of their wide range of pharmacological properties. The first bacterial type III PKS to be characterized, RppA, catalyzes the condensation of five malonyl-CoA molecules to produce 1,3,6,8-tetrahydroxynaphthalene, which serves as an intermediate in the biosynthesis of hexahydroxyperylenequinone melanin in the actinomycete Streptomyces griseus (Ueda et al. 1995; Funa et al. 1999). PhlD, a type III PKS in Pseudomonas fluorescens, is responsible for the biosynthesis of 2,4-diacylphloroglucinol, which has biocontrol activity against soilborne fungal plant pathogens (Bangera and Thomashow 1999). DpgA, a type III PKS from Amycolatopsis mediterranei, is involved in the biosynthesis of glycopeptide antibiotics such as vancomycin (Pfeifer et al. 2001). The discoveries of these type III PKSs have established the idea that type III PKSs are distributed widely not only in plants but also in microorganisms.

Based on bioinformatic predictions and subsequent in vivo and in vitro analyses, a number of type III PKSs have been shown to be responsible for the biosynthesis of various secondary metabolites, such as antibiotics and pigments. In addition, “steely,” a type I fatty acid synthase-type III PKS fusion enzyme from the social amoeba Dictyostelium discoideum, synthesizes a differentiation-inducing factor (Austin et al. 2006). Furthermore, a growing number of microbial type III PKSs have been reported to be involved in the biosynthesis of phenolic lipids such as alkylresorcinols and alkylpyrones. Phenolic lipids are distributed widely in bacteria, fungi, and plants (Kozubek and Tyman 1999; Stasiuk and Kozubek 2010). They consist of a polar aromatic ring and a hydrophobic alkyl chain. The amphiphilic nature of phenolic lipids contributes to the formation of a stable monomolecular layer. Phenolic lipids also exhibit antimicrobial and antioxidation activities. In this review, we primarily describe the phenolic lipid-producing type III PKSs in three microorganisms, Azotobacter vinelandii, S. griseus, and Mycobacterium smegmatis.
2 The *ars* Gene Cluster in *A. vinelandii*

*A. vinelandii* is a Gram-negative, nitrogen-fixing soil bacterium that differentiates into metabolically dormant cysts under adverse environmental conditions (Lin and Sadoff 1968). The central body of the cyst is surrounded by a thick membrane composed of a modified capsule (the intine) and a layered outer shell (the exine). Phenolic lipids, such as alkylresorcinols and alkylpyrones, are the major lipids in the cyst membrane (Reusch and Sadoff 1983). A search of a genome database predicted the presence of a gene cluster, named the alkylresorcinol synthesis (*ars*) gene cluster, which is required for the biosynthesis of these phenolic lipids. This gene cluster consists of two type III PKS genes, *arsB* and *arsC*, and two genes, *arsA* and *arsD*, together encoding a type I fatty acid synthase (Fig. 1a). In vitro studies showed that

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**Fig. 1** Phenolic lipids produced by type III PKSs in *A. vinelandii*. (a) Gene organization of the *ars* gene cluster. (b) Pathways for the biosynthesis of alkylresorcinols and alkylpyrones in *A. vinelandii*. (c) Proposed model for the transcriptional regulation of the *arsABCD* operon in *A. vinelandii*. Positive and negative controls are represented by *broken arrows* and *blunt-ended dotted lines*, respectively.
ArsA and ArsD synthesize an acyl starter substrate for ArsB and ArsC (Fig. 1b) (Miyanaga et al. 2008). ArsB synthesizes alkylresorcinols from an acyl starter substrate and three molecules of malonyl-CoA through a decarboxylative aldol condensation (Funa et al. 2006). In contrast, ArsC synthesizes triketide pyrones and tetraketide pyrones from an acyl starter substrate and two and three molecules of malonyl-CoA, respectively, through lactonization. An ars disruptant, which is unable to produce phenolic lipids, forms cysts with a disorganized exine, suggesting that phenolic lipids play a structural role in the cysts (Funa et al. 2006; Segura et al. 2009).

Biosynthesis of the phenolic lipids is elaborately controlled in A. vinelandii (Fig. 1c). Transcription of the ars genes is very low in exponentially growing vegetative cells and slightly increases (14-fold) during stationary phase, whereas it is induced 200-fold under an encystment-inducing condition (Segura et al. 2009). ArpR, a LysR-type transcriptional regulator, is the master regulator of the arsABCD operon; ArpR activates the transcription of the ars genes by binding the upstream regulatory region of arsA during encystment (Romero et al. 2013). Acetoacetyl-CoA, which is a precursor of poly-β-hydroxybutyrate, was shown to increase the binding affinity of ArpR to the arsA upstream regulatory region (Romero et al. 2013). Furthermore, the exogenous addition of acetoacetyl-CoA to the medium increased the transcription of the ars genes, as well as the synthesis of phenolic lipids. Taken together, acetoacetyl-CoA seems to act as a coinducer to enhance the transcriptional activation of the ars genes by ArpR (Romero et al. 2013). Meanwhile, the stationary-phase sigma factor RpoS is required for the transcription of arpR, and therefore RpoS is essential for the synthesis of phenolic lipids (Cocotl-Yañez et al. 2011; Romero et al. 2013). RpoS also controls the synthesis of other cyst components such as alginate and poly-β-hydroxybutyrate (Cocotl-Yañez et al. 2011). In addition, the nitrogen-related phosphotransferase system comprising the ElNtr, NPr, and EIIA_Ntr regulates the synthesis of phenolic lipids (Muriel-Millán et al. 2015). The non-phosphorylated EIIA_Ntr protein negatively affects the transcriptional activation of arpR by RpoS and also seems to control the expression of arpR at the posttranscriptional level. Very recently, another posttranscriptional regulation of arpR was reported in the Gac-Rsm system (Romero et al. 2016). The translational repressor protein RsmA negatively regulates the translation of arpR by binding its mRNA. The two-component response regulator GacA activates the transcription of the small RNA RsmZ1 that binds RsmA and counteracts its repressor activity.

3 The srs Gene Cluster in S. griseus

The genome sequence of S. griseus (Ohnishi et al. 2008) led to the discovery of a gene cluster, named the Streptomyces resorcinol synthesis (srs) gene cluster, which encodes another type III PKS, in addition to RppA. This gene cluster contains srsA, srsB, and srsC, which encode a type III PKS, a methyltransferase, and a hydroxylase,
respectively (Fig. 2a) (Funabashi et al. 2008). SrsA synthesizes alkylresorcylic acids from an acyl starter substrate, one methylmalonyl-CoA molecule, and two malonyl-CoA molecules through an aldol condensation (Nakano et al. 2012). SrsB catalyzes the decarboxylative methylation of alkylresorcylic acids to yield alkylresorcinol methyl ethers, and SrsC catalyzes the hydroxylation of alkylresorcinol methyl ethers to yield alkylquinones (Fig. 2b). Because alkylresorcinol methyl ethers and alkylquinones are detected mainly in the cell wall/membrane fraction of S. griseus, these phenolic lipids seem to be associated with the cytoplasmic membrane (Funabashi et al. 2008). An srs disruptant, which does not produce phenolic lipids, is highly sensitive to β-lactam antibiotics, including penicillin G, which is an inhibitor of peptidoglycan synthesis, which suggests that phenolic lipids confer penicillin
resistance on *S. griseus*. Phenolic lipids may affect the characteristics and rigidity of the cell wall/cytoplasmic membrane.

### 4 An Alkylquinone Biosynthetic Gene Cluster in *M. smegmatis*

*M. smegmatis* has an *srs*-like gene cluster that consists of a type III PKS gene (PKS10, MSMEG_0808), a methyltransferase gene (MSMEG_0809), and a hydroxylase gene (MSMEG_0811) (Fig. 2a). Recent studies have confirmed that this gene cluster is responsible for the biosynthesis of alkylquinones (Anand et al. 2015). PKS10 synthesizes alkylresorcinols from an acyl starter substrate, methylmalonyl-CoA, and malonyl-CoA. A *pks10* knockout strain, which does not produce alkylquinones, forms fragile biofilms, and it exhibits lower levels of ATP synthesis and higher levels of NADH accumulation, which are indicative of a disruption in the respiratory chain. In addition, alkylquinones were shown to function as electron carriers in the respiratory electron transport chain. Taken together with the fact that the expression of these genes is upregulated under oxygen-depleted conditions, these observations suggest that alkylquinones are necessary for maintaining cellular respiration in biofilms. These polyketide-type alkylquinones are biosynthetically distinct from canonical respiratory quinones that are derived from the shikimate and isoprenoid pathways.

### 5 Phenolic Lipid Biosynthetic Machinery in Various Bacteria

*srs*-like gene clusters, which contain a type III PKS gene, a methyltransferase gene, and a hydroxylase gene, are found widely among Gram-positive and Gram-negative bacteria (Fig. 2a). This fact implies that alkylquinone-type phenolic lipids are produced in various bacteria. For example, *Myxococcus xanthus* has the fatty acyl-AMP ligase-dependent type III polyketide synthase (*ftp*) gene cluster for phenolic lipid biosynthesis (Hayashi et al. 2011). The *ftp* cluster consists of a type III PKS gene (*ftpA*), a methyltransferase gene (*ftpB*), a stand-alone acyl carrier protein-encoding gene (*ftpC*), an acyl-AMP ligase gene (*ftpD*), and a hydroxylase gene (*ftpE*). *M. xanthus* seems to use two additional proteins, FtpC and FtpD, to produce the FtpA starter substrate. FtpA synthesizes an alkylresorcylic acid, which is modified by FtpB and FtpE to generate an alkylquinone, as occurs in the SrsABC reaction.

Some bacterial species contain an additional prenyltransferase gene in their *srs*-like gene cluster. *Actinoplanes missouriensis* has the alkyl-O-dihydrogeranyl-methoxyhydroquinone (*agq*) gene cluster, which consists of a type III PKS gene (*agqA*), a hydroxylase gene (*agqB*), a methyltransferase gene (*agqC*), and a prenyltransferase gene (*agqD*) (Fig. 2a). The prenyltransferase AgqD attaches a prenyl group to the C4-hydroxy group of the alkyl-methoxyhydroquinones, which are produced by AgqABC, to yield geranylated phenolic lipids (Awakawa et al. 2011).
Although the biological functions of the prenylated phenolic lipids in *A. missouriensis* remain elusive, they are assumed to have some important functions because *agg*-like gene clusters are conserved among some related actinomycetes, such as *Micromonospora aurantiaca* and *Salinispora tropica*.

In contrast, some *srs*-like gene clusters lack a hydroxylase gene (Fig. 2a). For example, the *Bacillus* pyrone synthase (*bps*) gene cluster in *Bacillus subtilis* consists of two genes: *bpsA* encoding a type III PKS and *bpsB* encoding a methyltransferase. In vivo and in vitro analyses showed that BpsA synthesizes triketide alkylpyrones from an acyl starter substrate and malonyl-CoA and that BpsB catalyzes the methylation of the alkylpyrones to yield alkylpyrone methyl ethers (Nakano et al. 2009). The *Rhodospirillum* polyketide synthase (*rps*) gene cluster in *Rhodospirillum centenum* also consists of only two genes: *rpsA* encoding a type III PKS and *rpsB* encoding a methyltransferase (Awakawa et al. 2013).

**Fungal and Plant Type III PKSs that Are Responsible for Phenolic Lipid Biosynthesis**

Filamentous fungi also contain type III PKS genes. A type III PKS from *Neurospora crassa*, 2'-oxoalkylresorcinolic acid synthase (ORAS), has been characterized (Funa et al. 2007). In vitro studies showed that ORAS has the ability to produce an alkylresorcylic acid from a long acyl starter substrate. However, *oras* gene deletions do not exhibit any apparent phenotypic changes. Moreover, production of the alkylresorcinol acid or the corresponding alkylresorcinol could not be detected in *N. crassa*, probably because only small amounts of these polyketides are produced.

In plants, phenolic lipids are also synthesized by type III PKSs. In *Sorghum bicolor*, two type III PKSs, ARS1 and ARS2, are involved in the biosynthesis of sorgoleone, which is an alkyquinone that is responsible for the allelopathic property of the plant (Cook et al. 2010). A methyltransferase (SbOMT3) and a hydroxylase (possibly a P450 monooxygenase) are also involved in sorgoleone biosynthesis (Baerson et al. 2008), as was observed for phenolic lipid biosynthesis in *S. griseus*. Rice (*Oryza sativa*) contains 31 type III PKS candidates. Among them, Os10g07040 (ARAS1) and Os10g08620 (ARAS2) were shown to catalyze the formation of alkylresorcylic acids (Matsuzawa et al. 2010). Bis-5-alkylresorcinols, which have a dihydroxybenzene ring at both terminal ends of an alkyl chain, have been isolated from some plants. Although in vitro studies suggest that bis-5-alkylresorcinols might be synthesized by type III PKSs, no genuine type III PKS has been identified to be responsible for the biosynthesis of bis-5-alkylresorcinol in any plant (Miyanaga and Horinouchi 2009).
7 Research Needs

Many type III PKSs have been discovered by genome mining. Some of the compounds that are synthesized by these type III PKSs exhibit important biological activities. Recent studies showed that some type III PKSs play important roles in the biosynthesis of biological membrane components and electron-shuttling molecules in bacteria. Further studies of other unexploited type III PKSs will identify additional novel biological components and bioactive substances.

References


